beta-Secretase Activity Assay Kit

Catalog Number KA0900

100 assays

Version: 04

Intended for research use only

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Introduction

Background

β-Secretase has been implicated to be an excellent target for anti-amyloid therapy for the treatment of Alzheimer's disease. The β-Secretase activity Assay Kit provides a convenient fluorescence method for detecting β-secretase activity in biological and purified samples. The assay utilizes a secretase-specific peptide conjugated to two reporter molecules EDANS and DABCYL. In the uncleaved form, the fluorescent emissions from EDANS are quenched by the physical proximity of the DABCYL moiety. Cleavage of the peptide by secretase physically separates EDANS and DABCYL allowing for the release of a fluorescent signal. The level of secretase enzymatic activity in samples is proportional to the level of fluorescence intensity.

General Information

Materials Supplied

List of component

Component	Amount
β-Secretase Extraction Buffer	25 ml
β-Secretase Reaction Buffer (2X)	10 ml
β-Secretase Substrate (in DMSO)	200 µl
Active β-Secretase (Lyophilized)	1 vial
β-Secretase Inhibitor (in DMSO)	10 µl

Storage Instruction

Store kit at -20℃.

Assay Protocol

Reagent Preparation

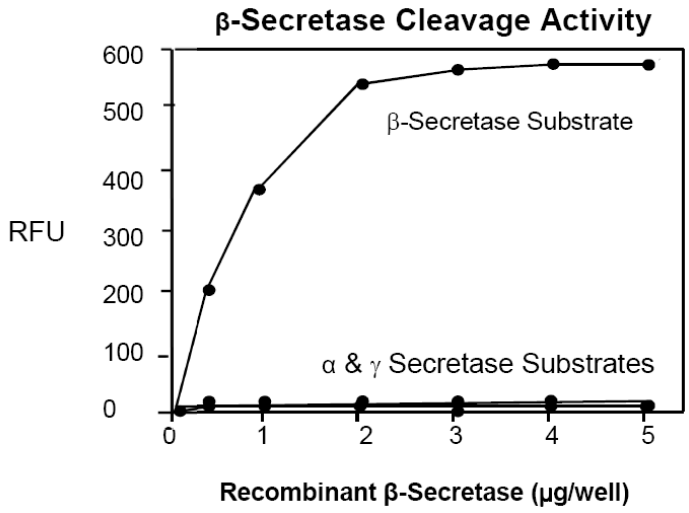
- ✓ Reconstitute the lyophilized Active β-Secretase by adding 10 µl of ddH_2O. The enzyme should be refrozen immediately at -70℃ after each use to avoid loss of activity. The enzyme is sufficient for 5 positive control assays (2 µl/assay).
- ✓ Assay can be performed directly in a 96-well white plate with flat bottom.

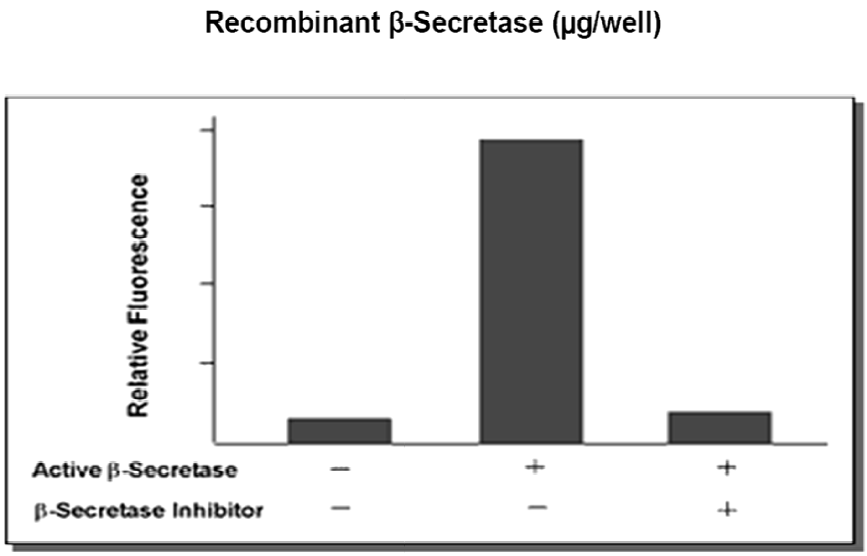
Assay Procedure

1. Collect cells (5 x 10^6 cells/assay) by centrifugation for 5 min at 700x g. Add 0.1 ml of ice-cold Extraction Buffer. For tissue sample, add 2-3X volume of ice-cold Extraction Buffer to tissue sample and homogenize it on ice.
2. Incubate cell lysate on ice for 10 minutes and centrifuge at 10,000x g for 5 minutes. Transfer the supernatant to a new tube and keep on ice. This should yield a lysate with a protein concentration of ~2-4 mg/ml.
3. Add 50 µl cell lysate (~2-5 x 10^6 cells or 25 - 200 µg of total protein) to each well in a 96-well plate.
   For positive control assay, add 2 µl of reconstituted Active β-secretase to 50 µl of Extraction Buffer.
   For negative control assay, add 2 µl of the β-Secretase Inhibitor to the 50 µl Sample or Positive Control well.
4. Add 50 µl of 2X Reaction Buffer.(if using inhibitor, gently mix then pre-incubate 5-10 min at 37℃ BEFORE ADDING SUBSTRATE)
5. Add 2 µl of β-Secretase substrate.
6. Cover the plate, tap gently to mix, and incubate in the dark at 37℃ for 1 hour.
7. Read sample in a fluorescence plate reader with Ex. = 335-355 nm and Em. = 495-510 nm.
   Background reading from substrate (without secretase) must be subtracted from all treated and untreated samples before calculating the fold increase in secretase activity *(Note: Background reading from substrate can be quite high, due to the nature of such fluorescence quenching assay.)*
   β-Secretase activity can be expressed as the Relative Fluorescence Units per µg of protein sample.
   Note: Recombinant β-Secretase exclusively cleaves β-Secretase substrate. It does not cleave α– or γ-Secretase substrates.

Data Analysis

Calculation of Results





Resources

Troubleshooting

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer datasheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly

Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range

Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.